Formulation and Evaluation of Niosomes of Valacyclovir Hydrochloride

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ABSTRACT
Valacyclovir hydrochloride niosomes were prepared by thin film hydration and ether injection process with different ratios of (1:2:2, 1:4:2) drug and and Span-80, Span 20 (Non-ionic surfactant) cholesterol. The niosomes prepared were in the size range of 0.5-5 microns in the case of hand shaking process and 0.5-2.5 microns in the case of Ether injection process. The order of encapsulation efficiency increases when span-80 concentration was increased. In-vitro release study on valacyclovir hydrochloride niosomes indicates 99.15% release for formulation prepared with cholesterol: Span-80 (2:4) and it takes an extended period of 1 day and 12 h for release.

Keywords: Gene, RAPD, antioxidants, antibacterial activity, Solanum nigrum, Fusarium.

ARTICLE INFO

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1. Introduction
Niosomes are multilamellar or unilamellar vesicles wherein an aqueous solution of solute(s) is enclosed in highly ordered bilayer made up of non-ionic surfactant with or without cholesterol and dicetyl phosphate and exhibit behavior similar to liposomes. The vesicles are formed on hydration of mixture of cholesterol and a single alkyl-chain non-ionic and non-toxic surfactant. Since then a number of non-ionic surfactants have been used to prepare vesicles.
vz. Polyglycerol alkyl ethers, glucosyl dialkyl ethers, crown ethers, ester-linked surfactants, polyoxyethylene alkyl ethers, and a series of brij, spans and tweens. Resultant vesicles have been termed niosomes. Non-ionic surfactants used in niosomes are non-toxic and non-irritant effects have been reported so far in animal studies due to the use of niosomes as drug carrier.

**Salient Features:**

1. Niosomes entrap solute in manner analogous to liposomes.
2. Niosomes are osmotically active and stable as well as they increase the stability of entrapped drug.
3. Handling and storage of surfactants require no special conditions.
4. Niosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubility’s.
5. Niosomes exhibit flexibility in their structural characteristics (composition, fluidity, size) and can be designed according to the desired situation.

**Types of Niosomes:**
The niosomes have been classified as a function of the number of bilayers (e.g. MLV, SUV) or as a function of size (e.g. LUV, SUV). The types of niosomes are:

1. Multilamellar Vesicles (MLV), SIZE >0.05 μ.
2. Small Unilamellar Vesicles (SUV), size 0.025-0.05 μ.
3. Large unilamellar vesicles (LUV), size>0.10 μ.
4. Valacyclovir Hydrochloride is 2-[(2-aminooxo-6,9-dihydro-3H-purin-9-yl)methoxy]ethyl (2S)-2-amino-3-methylbutanolate antiviral drug used in the management of herpes simplex, herpes zoster (shingles) and Bit is a prod rug, being converted in vivo to acyclovir.

**Advantages of Niosomes:**
The niosomal drug delivery is a potential drug delivery method for controlled and targeted drug delivery; the major advantages of these vesicular drug carriers are:

- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.
- Niosomes possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubility’s.
- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume, surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation.

**2. Materials and Methods**

Valacyclovir hydrochloride was purchased from yarrow chem. products, mumbai, India. Cholesterol, Span-80, span-20 was procured from Loba chemicals and S.D. Fine chemicals, Mumbai, India. Methanol, Merck-specialities private limited, Mumbai. Sodium hydroxide pellets Thermo fisher scientific india pvt.Ltd. Mumbai. Diethyl ether, Merck-specialities private limited, Mumbai. Potassium di hydrogen phosphate Merck-specialities private limited, Mumbai.

**Ether Injection Method:**
Drug, span 20 and span 80, cholesterol were taken in prescribed ratio (1:2:2, 1:4:2) in a 250 ml beaker. The mixture was dissolved in diethyl ether and methanol (8:2) solution and slowly injected through 14 gauge needle in to a beaker containing valacyclovir hydrochloride in 10 ml phosphate buffer pH 7.4 the temperature maintained during the injection was 40-600C. The difference in temperature between phases causes rapid vaporization of ether, methanol resulting in spontaneous vesiculation.

**Thin Film Hydration Method:**
Drug, span 20 and span 80 and cholesterol were taken in various ratios (1:2:2,1:4:2) and transferred in to a clean round bottom flask. Then diethyl ether and methanol(8:2) solution was added and the flask was fixed to rotary evaporator at 500C temp, for 20 mins under vaccum at 50 rpm. It forms a dry thin film along the sides of the R.B. flask. valacyclovir hydrochloride dissolved in phosphate buffer pH 7.4 and was added to the thin film and vortexed at room temperature for 20 mins which forms milky white suspension.
Table 1: Composition of niosomal formulations of valacyclovir hydrochloride by ether inject

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug</th>
<th>Span 20</th>
<th>Span 80</th>
<th>cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100mg</td>
<td>200mg</td>
<td>-</td>
<td>200mg</td>
</tr>
<tr>
<td>F2</td>
<td>100mg</td>
<td>400mg</td>
<td>-</td>
<td>200mg</td>
</tr>
<tr>
<td>F3</td>
<td>100mg</td>
<td>-</td>
<td>200mg</td>
<td>200mg</td>
</tr>
<tr>
<td>F4</td>
<td>100mg</td>
<td>-</td>
<td>400mg</td>
<td>200mg</td>
</tr>
</tbody>
</table>

Table 2: Composition of niosomal formulations of valacyclovir hydrochloride by thin film hydration method

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug</th>
<th>Span 20</th>
<th>Span 80</th>
<th>cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>100mg</td>
<td>200mg</td>
<td>-</td>
<td>200mg</td>
</tr>
<tr>
<td>F6</td>
<td>100mg</td>
<td>400mg</td>
<td>-</td>
<td>200mg</td>
</tr>
<tr>
<td>F7</td>
<td>100mg</td>
<td>-</td>
<td>200mg</td>
<td>200mg</td>
</tr>
<tr>
<td>F8</td>
<td>100mg</td>
<td>-</td>
<td>400mg</td>
<td>200mg</td>
</tr>
</tbody>
</table>

Characterization of Niosomes

Physical appearance of niosomal suspension:
The prepared niosomal suspension was viewed by naked eye to characterize color and physical state of suspension. Niosomal suspension was also viewed by optical microscope at 40 X magnification, to observe crystal characteristics of suspension by spreading a thin layer of niosomal suspension on a slide and placing the cover slip on it. The appearance for each formula was checked such as color, consistency and fluidity and comparison of each one with the other.

Vesicle Size Analysis:
Size and size distribution studies were done for niosomes. The suspension of niosomes was observed under optical microscope at 40x magnification. The sizes of 100 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope.

Vesicle morphology
Shape and surface morphology of niosomes was studied using scanning electron microscopy (SEM). The niosomes formed were mounted on an aluminum stub with double-sided adhesive carbon tape. The vesicles were then sputter-coated with gold/palladium using a vacuum evaporator and examined with the scanning electron microscope equipped with a digital camera at 25kV accelerating voltage.

Drug encapsulation efficiency:
Niosomal suspension (5ml) was placed in a glass tube. The aqueous suspension was sonicated in a sonicator bath for 15 min. The valacyclovir hydrochloride containing niosomes were separated from unentrapped drug by centrifugation at 13000 rpm at 20°C for 90 min. The supernatant was taken and diluted with phosphate buffer of pH 7.4. And the free drug concentration in the resulting solution was assayed by the UV spectroscopic method at 245 nm. The percentage of drug encapsulation was calculated by using the following equation.

\[ \text{EP} (\%) = \frac{(c_t - c_r)}{c_t} \times 100 \]

Where \( \text{EP} \) is the encapsulation percentage, \( c_t \) is the concentration of total drug, and \( c_r \) is the concentration of free drug.

In-Vitro diffusion study
The In-vitro release studies on niosomal suspension was performed using open ended tube which acts like donor compartment. The capacity of receptor compartment was 100 ml pH7.4 phosphate buffer placed in 250 ml beaker.. The membrane was mounted between the donor and receptor compartment. A weighed amount of niosomal suspension was placed on one side of the membrane. The receptor medium taken was 100 ml of phosphate buffer of pH 7.4.. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. The receiver fluid was stirred with a magnetic stirrer at a speed of 600 rpm. At each sampling interval, (1 ml) were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. The Sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectrophotometrically at 245 nm. The percentage drug release was calculated using calibration curve of the drug in phosphate buffer of pH 7.4.

Stability Studies:
Stability studies were conducted for best formulation of F4 of valacyclovir hydrochloride niosomal suspension. The ability of vesicles to retain the drug (Drug Retention Behavior) was assessed by keeping the niosomal suspension at three different temperature conditions, i.e., Refrigeration Temperature (4-8°C), Room Temperature (25±2°C) and oven (45±2°C). Throughout the study, niosomal formulation was stored in aluminum foil-sealed glass vials. The stored formulation was analyzed for particle size & percent drug entrapment at 245 nm.

3. Results and Discussion
The present investigation was carried out the on the formulation and evaluation of niosomes of valacyclovir hydrochloride were formulated using two different non-ionic surfactants (span 20, span80), and cholesterol. Diethyl
ether and methanol (8:2) was used as solvent, the phosphate buffer pH 7.4 was used as aqueous phase. Pre formulation studies were primarily done to investigate the physicochemical properties of drug and also to establish with non-ionic surfactants and other acceptance.

Compatibility studies:
From the FT-IR spectra of physical mixture of the drug, non-ionic surfactants, cholesterol, it was observed that the peak of major functional groups of valacyclovir hydrochloride which were present in the spectrum of pure drug were present below table and figure. The presence of peaks at were characteristics to the pure valacyclovir hydrochloride. IR spectrum of physical mixture of optimized formulation of drug revealed that there was no appreciable change in position and intensity of peak with respect to IR spectrum of pure valacyclovir hydrochloride. IR analysis revealed that was no known chemical interaction between drug and surfactant, cholesterol.

### Table 3: Functional groups of pure valacyclovir hydrochloride

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Wave number cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O(str)(1735-1750)</td>
<td>1738.47</td>
</tr>
<tr>
<td>C-O (str)(alcohol 1250-1350)</td>
<td>1296.92</td>
</tr>
<tr>
<td>C=O(str)(phenols 1310-1410)</td>
<td>1365.99</td>
</tr>
<tr>
<td>C=C(str)(alkene 1680-1620)</td>
<td>1632.64</td>
</tr>
<tr>
<td>C=C(str)(aromatic 1450-1600)</td>
<td>1454.84</td>
</tr>
</tbody>
</table>

**Vesicle morphology:** The morphology of niosomes derived from proniosomal gel was studied using scanning electron microscopy. SEM images of valacyclovir hydrochloride revealed that the niosomes were spherical in shape and discrete with sharp boundaries having large internal aqueous space. SEM images of niosomes produced from optimized formulation F4, F8 shows in the optical microscopy images of the niosomes from different formulation are shown in.

**Vesicle Size Analysis:**
The vesicles size of valacyclovir hydrochloride niosomes are present in which indicated that vesicles formed with span 20 is smaller in size than vesicles formed with span 80. This is due to greater hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity, resulting in smaller vesicles. This would also explain the large vesicles size of niosomes prepared with span 80 which has a much lower hydrophobicity than does span 20. Vesicles prepared by thin film hydration method were small in size compared to vesicles prepared with ether injection method, due to rotation applied during thin film hydration method.
Comparison of Vesicles Size

Drug encapsulation efficiency:
Encapsulation efficiency of niosomes formulations ranged from the drug encapsulation efficiency of all eight formulations are shown in table 6.8 and fig 6.8. As shown in table, encapsulation efficiency of niosomes formed from span 80 was found high compared with niosomes prepared from span 20. Niosomes prepared by ether injection method were having greater encapsulation efficiency compared to niosomes prepared by thin film hydration method. Most of the surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. As the surfactant content of the formulation increased, the encapsulation of drug also increased. The formulations (F1-F4) contain 1:2:2, 1:4:2 ratio of drug: surfactant: cholesterol prepared by ether injection method shows high encapsulation efficiency compared to the formulations (F5-F8) contain 1:2:2, 1:4:2 ratio of drug: surfactant: cholesterol prepared by thin film hydration method.

In –Vitro Diffusion Study:
The niosomal formulations of valacyclovir hydrochloride (F1-F8) were characterized for their drug permeation study sing open ended tube through an artificial membrane. Drug diffusion study of all the formulations was carried out sing phosphate buffer of pH 7.4 for 12 hrs at 37±0.5ºc with 500 rpm speed. Samples were withdrawn at regular intervals (0,1,2,3,4,5,6,7,8,9,10,11,12h). At every interval, 5ml of sample was withdrawn. After appropriate dilution the sample solution were analyzed at 245 nm for valacyclovir hydrochloride by sing UV-visible spectrophotometer.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Cumulative percentage of drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>F1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>8.96±0.31</td>
</tr>
<tr>
<td>2</td>
<td>14±0.26</td>
</tr>
<tr>
<td>3</td>
<td>16.56±0.89</td>
</tr>
<tr>
<td>4</td>
<td>18.01±0.31</td>
</tr>
<tr>
<td>5</td>
<td>29.36±0.54</td>
</tr>
<tr>
<td>6</td>
<td>39.36±0.13</td>
</tr>
<tr>
<td>7</td>
<td>49.36±0.14</td>
</tr>
<tr>
<td>8</td>
<td>58.36±0.15</td>
</tr>
<tr>
<td>9</td>
<td>69.02±0.52</td>
</tr>
<tr>
<td>10</td>
<td>75±0.31</td>
</tr>
<tr>
<td>11</td>
<td>86.35±0.26</td>
</tr>
<tr>
<td>12</td>
<td>95.45±0.23</td>
</tr>
</tbody>
</table>

All values represent mean standard deviations (SD) n=3

The cumulative percentage of valacyclovir hydrochloride released from noisome formulations containing different non-ionic surfactants was reported in above table. The result indicate that the noisome with increased ratio of surfactant have high release rate. The percent cumulative release in 12hrs in above table was found to be in the range of 93.36%-99.15%. From the in vitro release data, it may be concluded that the formulations F4 (contain drug, span 80, (non-ionic surfactant), cholesterol in 1:4:2 ratio) prepared by ether injection method was selected as best formation as the drug release from these formation was 99.15% in 12hrs and showed optimum physic chemical properties.
Cumulative percent drug release profile of valacyclovir hydrochloride niosomal formulations (F1-F2-F3-F4)

The release data in first order and zero order, the $R^2$ values of all the formulations greater for zero order model. So all the formulations in this study were best expressed by zero order classical diffusion equation. The linearity of plot indicated that the release process was diffusion controlled. To further confirm the exact mechanism of drug release, the data was incorporated into Kores Meyer Peppas model and the mechanism of drug release was indicated according to the value of exponent ‘$n$’ value found to be between 0.5 to 0.89, so it indicates all the niosomal formulations followed non-fickian diffusion.

Stability studies:
The niosomal dispersion showing highest entrapment efficiency (F4) was stored in three different temperatures 4±2ºc, 25±2ºc, 45±2ºc. The encapsulation efficiency and vesicle size was again calculated after 2 months.

4. Conclusion
Valacyclovir hydrochloride was successfully encapsulated into niosomes by thin film hydration method and ether injection process. Could be used as a drug carrier for valacyclovir hydrochloride, for producing prolonged activity and simultaneously reducing side effects. Drug incorporation in the niosomes to target the niosomes to the specific site is a promising drug delivery model. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposome’s, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure. Niosomes are considered to be better candidates for drug delivery as compared to liposome due to various factors like cost, stability etc. Niosomes are promising...
vehicles at least for lipophilic drugs. These advantages over the liposomes make it a better targeting agent. Ophthalmic, topical, parental and various other routes are used for targeting the drug to the site of action for better efficacy.

5. References